BIOLOGICAL NITROGEN FIXATION—EARLY AMERICAN STYLE[1]

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Establishment of the State Agricultural Experiment Stations

The year 1962 marked three noteworthy anniversaries that have been observed with appropriate exercises, conferences, publications, and similar events at Land Grant colleges throughout the nation. These were: (i) the centennial of their authorization by the Morrill Act of 1862; (ii) the centennial of the establishment of the United States Department of Agriculture; and (iii) the 75th anniversary of the passage of the Hatch Act, the basic legislation for the State Experiment Stations. Since the American Society for Microbiology has a Division of Agricultural and Industrial Bacteriology, it seems appropriate that notice, even though somewhat belatedly, be taken of these far-reaching legislative events. My contribution, therefore, is dedicated to those pioneers, some of whom were members of this Society, whose careers in the early days of these institutions helped establish the patterns of today's research.

In 1862, agricultural experimentation was not unknown in the United States, but its typical expression was that of the Trustees' garden in Georgia established in 1733 (14), rather than that of the modern experiment station. Among the many individuals who contributed to this change, two of the leaders were S. W. Johnson, professor of chemistry at Yale (Sheffield), and his protege W. O. Atwater (Fig. 1).

Johnson had returned to Yale in 1855 after spending a few years in Germany where he had been deeply impressed with the operation of the agricultural experiment stations, especially the one at Moeckern. His efforts to set up a similar station in Connecticut were delayed because of the Civil War, but by the seventies he had clearly in mind the requirements of an "ideal" station. These were: (i) it should have a State charter and subsidy, (ii) its sole function should be research, and (iii) it should be near to but independent of a college (15, p. 22). In addition, Johnson proposed what must have been a real shocker to those whose idea of an experiment station was that it should be concerned almost exclusively with variety trials of crops and chemical analyses of soils and fertilizers. He stated that the station should be in a suburban area rather than on a farm. Johnson's reasons for this choice were that it was important to be near facilities of library, post, telegraph, and express office, as well as public utilities; moreover, the experimental work could be more effectively carried out in laboratory, greenhouse, and small garden plots than in the field. Through the influence and subsidy of Orange Judd, farm paper publisher and former student of Johnson, a trial station was authorized to be set up at Wesleyan University with W. O. Atwater as its director. Apparently, Atwater yielded nothing to his former teacher in his zeal for the "ideal station"; it was written of him (15, p. 23):

> The Johnsonian formula for station organization owed its triumph in no small way to the evangelical efforts of the young chemist, Wilbur Atwater, who in 1873 had begun a professorial career at Wesleyan after earning his doctorate in Johnson's laboratory in 1869 and spending 2 additional years in post-graduate work at German experiment stations. Filled with a ministerial zeal for spreading the gosepl of German agricultural science, Atwater volunteered his pen and his voice in the station campaign in Connecticut. Energetically in the midseventies he publicized his first hand knowledge concerning the work and current organization of the German

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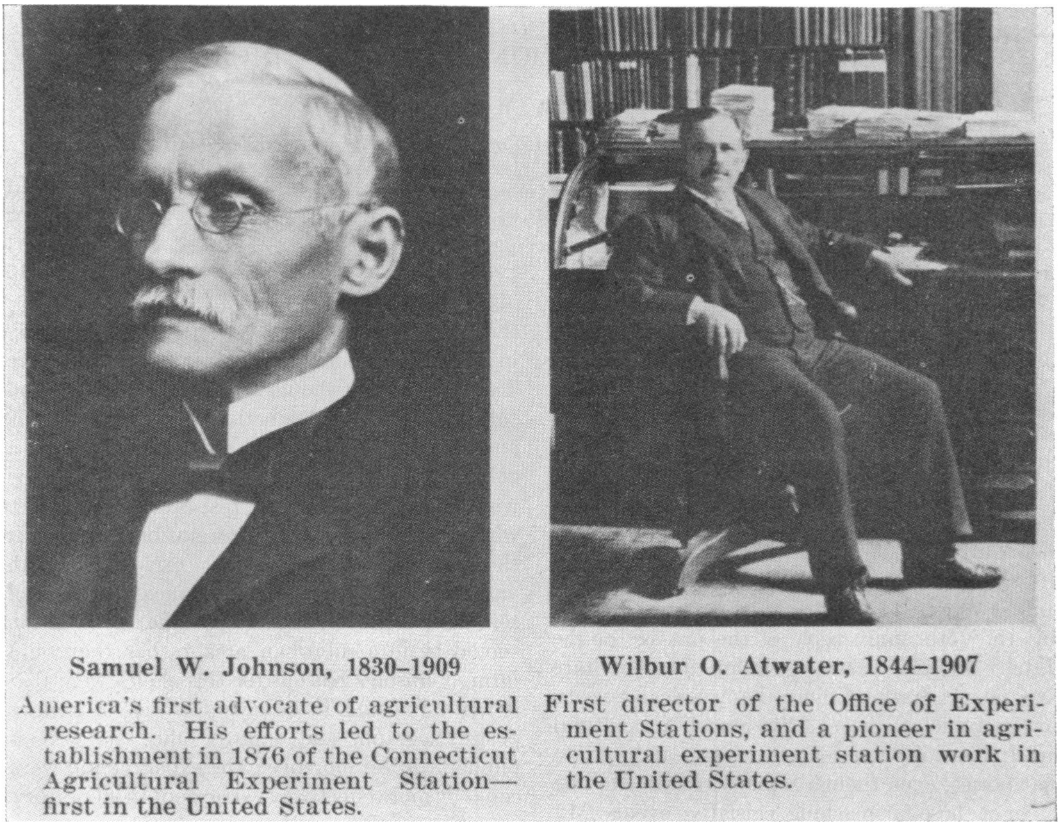

FIG. 1. *Portraits of Johnson and Atwater. From Knoblauch, Law, and Meyer* (15). *This book is an interesting and authoritative history of the state experiment stations written to commemorate the 1962 anniversaries of the Land Grant and Hatch Acts.*

> stations. His definition, a product of his recent apprenticeship at the German stations, coincided with Johnson's and underlined the central significance of basic research.

Note that this trial station was not yet a full fledged State experiment station, since its support initially was both public ($2800 annually) and private (Judd gave $1000 a year and Wesleyan furnished quarters). But 2 years later (1877) the Connecticut State Experiment Station was formally set up with temporary quarters near Sheffield and with S. W. Johnson as its director.

The next decade witnessed widespread agitation in the form of meetings, memorials to Congress and state legislatures, and articles in the public and agricultural press advocating the establishment of state stations. Progesss was slow, primarily because of difference of opinion among the advocates. Some states had followed Connecticut's lead in establishing independent stations; others, including eight of the states with land grant colleges, had placed them under college administration. Also, the question of financing was touchy—advocates vaguely suggested that funds be provided by private agricultural organizations, by the states, and also by the Federal government. Opponents bridled at the latter proposal asking (15, p. 42):

> Why, for example, should Congressmen, acutely aware of the historic distinctions between Federal powers and States' rights, intervene in the responsibilities of the State governments in order to do what the State legislatures had not done? Why should Congressmen, politically accustomed to a Federal Government of strictly limited

> functions, activate at this time, the rudimentary precedents which for 20 years had lain dormant? If, furthermore, American farmers desperately needed the benefits of experimentation, why had not the farmers, comprising a substantial majority of the American citizenry, insisted long since that stations be established?

The reply was made by E. W. Hilgard, director of the California station at Berkeley; writing to Johnson in March, 1882, he stated, "I am beginning to feel aggressive on the subject," and in May he published an article in the *Atlantic Monthly* which presented cogent arguments for Federal subsidy (15, p. 41):

> The Federal Government, Hilgard urged, ought no longer to leave the problem of collegiate agricultural research solely to the jurisdiction of the individual State legislatures. It should recognize an unfortunate situation of nationwide dimension, namely, that the States most in need of the improved technology that only a local station could provide had stingy legislatures and penniless colleges. The Federal Government, then, should follow a corrective policy of "enlightened intervention" and supply "substantial aid" to each land grant college for the specific purpose of operating a station.

Finally, after much debate and political maneuvering, appropriate bills were introduced in Congress—and failed to pass. Eventually, one, the Hatch Act, became law but not without struggle and compromise. One amendment provided that the station need not be set up at an agricultural college. This was to gain support from those states which had already set up independent stations. For this writer, the unkindest cut was that Senator Spooner of Wisconsin introduced this provision on the grounds (23, p. 129):

> That the agricultural department of the University of Wisconsin had not been a success, and that it was almost impossible to secure the attendance of any larger number of agricultural students in classical institutions where from 300 to 500 students were pursuing classical or scientific courses.

Another amendment provided that a state need not participate if it did not wish. This solicitous attempt to protect states from federal subsidy proved unnecessary; in the first year, all states and one territory approved the provisions and duly collected the appropriation.

Although the Hatch Act did not embody all the Johnson-Atwater formulae for the "ideal" station, the really important one, that the stations should undertake basic research, was preserved.

Atwater's Experiments on Nitrogen Fixation at Wesleyan

In 1885, Atwater published results in the *American Chemical Journal* of the first experiments on biological nitrogen fixation undertaken in the United States. Before looking at these results, a few words about the paper itself are of interest. First, it is lengthy (21.5 pages) and somewhat repetitious. The essential data can be summarized in a single table, but many other tables are included giving details of the analyses, weight of seed, and other statistics together with alternative methods of calculating the results. On reading through these details, one finds less objectionable the modern practice of editors of demanding concise presentation. Second, it qualifies as a report of authentic research in this field as the experiments start with an accident that casts doubt on the findings. Atwater (4) explains:

> Besides the Nos. A, B and C, the series included two others, D and E, into which, unfortunately, a quantity of rain-water fell through a leak in the roof of the greenhouse during a violent shower. The water percolated through the sand and was caught in the beakers below. The accident was discovered by the gardener who had been charged to watch the plants, and who poured the contents of the beakers back upon the sand before either the assistant in charge of the experiments or myself had learned of the matter. One of the pots received more rain-water than the other, and more fell into the beaker below. The analysis at the end of the experiment showed in one case a loss of nitrogen and in the other a very large gain. . . .

> It would, of course, be unwarrantable to include these experiments with those described above (A, B and C), and, for that matter, the possibility of such an accident casts a suspicion upon the whole series, so that their results can be entitled to full credence only on condition of their being verified by repetition under circumstances fitted to preclude such sources of error.

Finally, the urbanity of the acknowledgements at the end should not be lightly dismissed in these days of sterile forms that "this research was supported by grant number such and such from the NSF or the NIH." Space is not at a premium, so Atwater writes:

> I improve this opportunity to express my obligations to my assistant, Mr. C. D. Woods, by whom the details of the experiments above described have been most faithfully and skillfully performed.
>
> It gives me a great pleasure, also, to bear testimony to the generosity of Hon. J. W. Alsop, M.D., of this city (Middletown, Conn.), who has, by defraying the larger part of the pecuniary cost, and by aid in other ways, made the investigations practicable.

The results shown in Fig. 2 leave little doubt but that Atwater obtained nitrogen fixation by the pea plants, nor does he hedge unduly on this aspect. He states forthrightly in his conclusion:

> The outcome of these experiments may be concisely stated thus: 1. The plants, peas, grown in nutritive solutions exposed to the air, but protected from rain and dew, contained at maturity much more nitrogen than was supplied them in nutritive solution and seed. Such were the results of a first series of trials, confirmed, even more strikingly by a second series the succeeding year. For this excess of nitrogen there was but one possible source, namely, the atmosphere.

He observes, however, "That the plants assimilate free nitrogen is contrary to general belief and to the results of the best investigations of the subject. The disagreement between these results and my own may, however, be more apparent than real. Two points in particular seem to me worthy of note."

The two points were: (i) since the negative experiments were made with little or no added combined nitrogen available to the plants, this might have depressed nitrogen fixation; and (ii) the most decisive experiments were conducted under glass covers connected with the earth, a circumstance that probably would eliminate a necessary electric potential. This latter novel explanation was based on the following consideration:

Statistics of Experiments on the Assimilation of Atmospheric Nitrogen by Peas.

Conditions of Experiments.			No. of Experiment.	Nitrogen supplied. In seeds.	Nitrogen supplied. In Nutritive Sol'n.	Nitrogen supplied. Total.	Nitrogen found at End of Experiment. In Plants.	Nitrogen found at End of Experiment. In Residual Solut'n.	Nitrogen found at End of Experiment. Total.	Apparent Gain + or Loss — of Nit'g'n
				Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.
Larger Nitrogen Ration.	Concentrated Solution.	Scantily fed.	8	70.3	136.9	207.2	197.5	12.7	210.2	+ 3.0
		Well fed.	6	34.8	136.9	171.7	149.6	1.2	150.8	— 20.9
	Dilute Sôlution.	Scantily fed.	12	68.8	136.9	205.7	260.2	45.7	305.9	+100.2
		Well fed.	10	34.6	136.9	171.5	277.8	35.7	313.5	+142.0
Smaller Nitrogen Ration.	Concentrated Solution.	Scantily fed.	7	71.5	59.4	130.9	158.1	0.0	158.1	+ 27.2
		Well fed.	5	34.2	59.4	93.6	156.1	0.0	156.1	+ 62.5
	Dilute Solution.	Scantily fed.	11	72.5	59.4	131.9	210.9	2.7	213.6	+ 81.7
		Well fed.	9	35.3	59.4	94.7	186.5	1.4	187.9	+ 93.2

FIG. 2. *Atwater's table summarizing the results of the first series of experiments (1885–1886)*

> Berthelot has shown that free nitrogen may be assimilated by vegetable substances, dextrine, cellulose, etc., under the influence of electricity of a potential similar to that which obtains near the surface of the earth, in the strata of air in which our cultivated plants grow. The inference that the compounds in living plants may assimilate nitrogen in the same way is natural. That this electrical tension may have been absent in the experiments with the plants under glass, referred to, would seem probable. The hypothesis of the assimilation of free nitrogen by plants through the agency of electricity, and the absence of that agency in the experiments of Boussigault and of Lawes, Gilbert and Pugh, would, with the effect of scanty food supply, explain the discrepancy between their experiments and my own, in which the conditions of growth were normal, and would clear up the worst difficulty in the much vexed question of the sources of nitrogen of plants.

In 1886, Atwater (5) returned to the argument in a long paper in which he compared his own results, including some new data dealing with liberation of nitrogen from nitrate by germinating seeds (probably bacterial denitrification), with "the best investigations." The chief interest of this paper is that a new factor is offered to join the other two in explanation of the discrepancies—the possible role of bacteria is recognized.

> To recapitulate in a few words: The experimental testimony regarding the acquisition of atmospheric nitrogen by plants is conflicting. But the evidence against it which comes from the laboratory and the greenhouse is based upon experiments whose conditions were more or less abnormal in respect to food-supply or access of nitrogen compounds or otherwise. In those which seem most conclusive against the assimilation of free nitrogen the arrangements were such as may have hindered the action of electricity, if not that of nitrogen-fixing microorganisms, two agencies toward which late research points as possible, if not certain, factors in the fixation of nitrogen. In all there is the possibility, and in some a very strong probability, that the results may have been affected by liberation of nitrogen from seeds or plants or food supplied, a liberation which is sometimes, if not always, due to ferments. This may materially reduce the nitrogen found at the end of the experiments, and with it the apparent gain of nitrogen from the air.

The inability of Atwater to choose between the physical and the biological explanations probably reflects his training and experience as a physical scientist. The published details of his chemical analyses can evoke our admiration today. But one wonders, admittedly fruitlessly, if Atwater ever discussed his experiments with his colleague in biology, young H. W. Conn, who was beginning to show interest in the role of "germs" in agricultural science.

On May 18, 1887, the Connecticut Assembly passed a resolution accepting and assenting to the provisions of the Hatch Act, and assigning one-half of the funds to the Storrs Agricultural School to be used for the experimental farm and the remaining one-half to the New Haven Station. Atwater was made the first director of the Storrs Station. At this time, Storrs was only a secondary school for boys; the 2-year program included such subjects as general and agricultural chemistry, natural philosophy, botany, zoology, mineralogy, surveying, theoretical agriculture, stock breeding, and English composition. Obviously, it was no "Do-the-Boys-Hall," but the Squeers' philosophy for educating the young was not overlooked. The teen-agers were also expected to "acquire dexterity" on the farm by laboring for 3 hr a day in the fall semester, and 5 hr each day in the spring. This work was supervised by a farm superintendent who had held a similar position for 27 years at the State reform school (22, p. 323). It seems a safe conclusion that, however much the boys might have neglected their homework, the field work was attended to with diligence.

In the second annual report (6), Atwater and Woods published further experiments on nitrogen fixation explaining:

> The circumstances which caused the cessation of the investigation from

1883 to 1885 were alike operative from the latter time until 1888, when the organization of the chemical work of the Station in the chemical laboratory of Wesleyan University, where the previous investigations had been made, rendered their continuation possible. Meanwhile the very important investigations of Hellriegel and others had confirmed those of the first and second series referred to, and, while bringing additional evidence of the acquisition of atmospheric nitrogen, had also indicated the very strong probability that microorganisms are active agents in the assimilation of nitrogen.

The results summarized in Fig. 3 are clear-cut and complete; this time the table includes information on the nodules, or "tubercles." The role of these is emphasized in the summary.

Peas, alfalfa, serradella and lupine certainly, clover in all probability, cow peas presumably, and perhaps leguminous plants in general, are able to acquire large quantities of nitrogen from the air during their period of growth.

That there is a connection between root tubercles and the acquisition of nitrogen is clearly demonstrated. What this connection is, what are the relations of micro-organism to the root tubercles and to the acquisition of nitrogen, and in general how the nitrogen is obtained, are questions still to be solved.

If brief, the acquisition of large quantities of atmospheric nitrogen by leguminous plants which was demonstrated by the former experiments here and has been since confirmed by Hellriegel and others, is still further confirmed by the experiments herewith reported. These experiments in like manner confirm the observation by Hellriegel of the connection between

EXPERIMENTS WITH PEAS AND ALFALFA, 1888–1889.

Condensed Summary of Results and Averages.

	Root Tubercles.	Without Nitrogen in Nutritive Solutions.			With Nitrogen in Nutritive Solutions.			Total.		
		No. of Experiments.	No. of Plants in Experiments.	Gain (or Loss) of Nitrogen.	No. of Experiments.	No. of Plants in Experiments.	Gain (or Loss) of Nitrogen.	No. of Experiments.	No. of Plants in Experiments.	Gain (or Loss) of Nitrogen.
Peas.	None,	10	23	—9.6	6	13	—22.7	16	36	—14.5
	Few,	7	21	—2.1	12	39	0.6	19	60	—0.4
	Fair number,	4	15	63.3	7	24	28.4	11	39	41 1
	Large number,	10	39	77.9	18	69	99.5	28	99	91.7
Alfalfa.	None,	—	—	—	—	—	—	—	—	—
	Few,	3	4	84.0	—	—	—	3	4	84.0
	Fair number,	—	—	—	1	3	137.5	1	3	137.5
	Large number,	1	1	382.2	—	—	—	1	1	382.2

FIG. 3. *Results of the second series of trials (1888–1889)—post Hellriegel and Wilfarth*

> root tubercles and the acquisition of nitrogen.

The last statement appears redundant and somewhat weak. Although Atwater himself probably took an active part in the experiments of 1888, those in 1889 must have been primarily under the direction of Woods who was now the acting director of the station. On October 1, 1888, Atwater left for Washington to become the first Director of the newly formed Office of Experiment Stations of the USDA.

Contributions from the Experiment Stations

Although Atwater must have been disappointed that he missed the essential observation that would have given him priority, the many contributions of the State Experiment Stations in the next decade to this field should have been a source of satisfaction. In an 1893 review of papers published during the past 2 years, Schneider (21) stated:

> I shall review foreign and American investigations separately, as the plans and ends sought for are somewhat different. European investigators have discovered the subject in hand and have studied it from a thoroughly scientific standpoint. Americans have but very recently taken up the subject and have treated it mainly with a view to turning it to some practical use while they yet, in many cases, lack sufficient scientific data.
>
> Since foreign investigators have made the most important investigations and have, so to speak, laid the foundation upon which future workers are to build, it is but just to give them our attention first.

His first citation of "the most important investigations" proved to be unfortunate—it was one by Frank and Immendorff that allegedly demonstrated that "all plants are capable of utilizing more or less of the free nitrogen of the air." Their conclusion on another controversial aspect, however, has stood the test of time.

> The question whether the rhizobium has in itself the power to assimilate free nitrogen is not fully demonstrated. From observations upon rhizobia in cultures, it seems that they do not assimilate more free nitrogen than other bacteria. It is most likely that the presence of the rhizobium in plants only awakens latent assimilating powers.

Considering their basic nature, the cited American contributions needed no apology. Schneider explained that in July, 1892, he had suggested that the rhizobia might be divided into several species. Having admitted that this was a bold and somewhat unwarranted thing to do (he did not say why), he went on to discuss a paper that Bolley later in that year contributed to the program of the Association of American Agricultural Colleges and Experiment Stations titled, "Notes on root tubercules (Wurzelknöllchen) of indigenous and exotic legumes in virgin soil of the Northwest." It certainly impressed Schneider who wrote:

> I wish to state here that the paper is remarkable, not so much for its scientific value, as for its exact and clear cut statements. They are the statements of a man whose preceptive faculties are wide awake and who can describe explicitly what he sees. It is sincerely to be hoped that more writers will follow his example.

Perhaps the source of his enthusiasm can be found in the following statement.

> His general conclusions are that tubercle-forming organisms are freely distributed through virgin soil, thus infecting legumes wherever they may be found. He also makes the suggestion that there may be more than one species of Rhizobium.

He is less satisfied with the extensive studies just published by Atkinson observing,

> His historical review is quite complete and of great importance to those who can read only English. The original part of his paper is rather unsatisfactory in many respects.

The final part of the paper is a summary of some experiments that Schneider was making at the Illinois Experiment Station. He describes an attempt to infect maize by cultivating rhizobia on an infusion containing extracts of corn roots. This was not successful, although he claims the organism did invade the root hairs of the corn.

Other trials were concerned with the physiological properties of the rhizobia and the morphology of the root nodules. Surprisingly, he stated "that it is very difficult to obtain pure cultures of any given species of rhizobia." Mention should also be made of two useful contributions of this early American period: the review by Atkinson (2) of the Station at Auburn, Ala., contained in a bulletin devoted to nematode rootgalls, and a bibliography of over 600 titles by MacDougal (16) of Minnesota.

Although the experiments discussed by Schneider certainly could qualify for a basic research grant from a federal agency today, it is true that workers at many of the stations concerned themselves with very practical questions such as the one forthrightly asked by Duggar (10): "What classes of soils in Alabama would be benefitted by inoculation for clover, vetch, etc.?" Stubbs (9), in a letter to the Commissioner of Agriculture and Immigration at the Baton Rouge Station in La., was more eloquent.

> DEAR SIR—The leguminous plants have been known to be restorative in their character when used for improvement of soils in a systematic rotation of crops. Some years since it was discovered that the chief virtue of these plants in abstracting and appropriating nitrogen from the air, was due to the tubercles which occur upon their roots. With the view of throwing light upon this subject, and especially of studying these leguminous plants which are in common use for soil restoration in this State, the following experiments were inaugurated, and have been successfully conducted by Prof. W. R. Dodson, Mycologist of the Station. For the purpose of diffusing information upon this subject among our farmers and planters, I ask that you publish this report as Bulletin No. 46.

Probably Bulletin 96 from the Kansas station at Manhattan, dealing with the inoculation of soybeans, typifies the research (8). It has an interesting table summarizing replies from state stations to an inquiry asking whether soybeans in the region possessed nodules. Six, including the Hatch Station at Amherst, Mass., reported that the organisms were indigenous to the soil: two said they had obtained nodules by inoculation: five could find no nodules; ten stated that they didn't grow soybeans (Minnesota and Washington explaining it was too cold in their regions), and eighteen hadn't looked. The Kansas workers imported some soil from the Hatch station and made some interesting greenhouse and field experiments from which they concluded:

> Our experiments have not been conducted long enough to thoroughly test the matter, but it is probable that a field once inoculated will always remain inoculated, and that the bacteria will slowly increase in the soil. The bacteria live for a long time in the soil after the plants are removed. We have kept dry inoculated soil in sacks two years, where it became as dry as road dust, and it had full strength in producing root tubercles when used.

After the turn of the century, many stations published similar bulletins; my last choice for examination is one at the federal level, U.S. Department of Agriculture Bureau of Plant Industry Bulletin 71, which is certainly a mixed bag (17). George T. Moore, its author, provided a valuable and scholarly historical account of the use of artificial inoculation, directions for use of an inoculum put out by the U.S. Department of Agriculture, and reports from satisfied customers. The names of Hellriegel and Wilfarth are consistently misspelled, and the statement is made that, when the organism is grown on a medium high in fixed nitrogen, it loses the power to fix atmospheric nitrogen and "for this reason the mere matter of an abundant growth is one of the least desirable considerations in propagating these organisms for any practical purpose." Moore also stated that he obtained significant fixation of nitrogen by the organism alone so that "there could be little doubt about the power of *Pseudomonas radicicola* to fix nitrogen independent of any leguminous plant." Even more interesting, he includes a photograph showing excellent growth of inoculated alfalfa without nodules, a result that he ascribes to "internal infection."

Since dirt farmers were not likely to be interested in the scientific portion of this bulletin, a companion Farmers' Bulletin 214 (18) was issued; it was strictly business, and was illustrated with line drawings to insure that the directions were understood (Fig. 4). This was essential,

FIG. 17.—Stirring seed moistened with culture liquid to hasten drying.

FIG. 4. *Illustration from Farmers' Bulletin 214*

since the method advocated was a do-it-yourself deal in which the farmer was furnished packaged constituents for adding to water to grow the bacteria which were supplied dried on cotton (U.S. Letter Patent 755,519). This bulletin also carried testimonials from farmers living at such nostalgia-invoking place names as Sandy Run, S.C., Grandview, Tenn., and Simplicity, Va. Adolph Soderberg of Sister Bay, Wis. reported that "there were more pods on the vines that were treated and about 3 bushels more peas to the acre on those that were treated," as well as "about twice as many nodule formations." An account of a remarkable experiment by one of the editors of *Hoard's Dairyman* (Fort Atkinson, Wis.) includes the following statement.

> Our field already shows the good effect of inoculation. (The method consisted in going over an alfalfa field that was not thriving with a sprinkling cart containing the culture liquid. . .)

Surprisingly, in view of their later interest in biological nitrogen fixation, bacteriologists at the station at Madison, Wis., did not contribute to the early publications. H. L. Russell, later Director of the Station, published one of the early reviews (19) on the subject, but a summary of work by the department (1893 to 1903) listed no experimental work in this field (20). An examination of bachelor of science theses in the E. G. Hastings Memorial Library of the department revealed that in the period from 1895 to 1915 only one dealt with nitrogen fixation, ranking this subject with "A study of the bacterial contamination of telephone transmitters" as the least popular areas for thesis research. The student, H. A. Smythe, noted:

> As the Bacillus Radicicola had never been grown in this laboratory, my first

> object was to secure the organism in pure culture on artificial media. This I found a much more difficult proposition than I had anticipated.

The 22nd Annual report of the station (1905) summarized some inoculation experiments on alfalfa and soybeans made by Russell and R. A. Moore in Agronomy. Conrad Hoffmann, the soil bacteriologist in the department, published with B. W. Hammer a research bulletin in 1910 dealing with nitrogen fixation by the azotobacter, but no consistent program in this field was

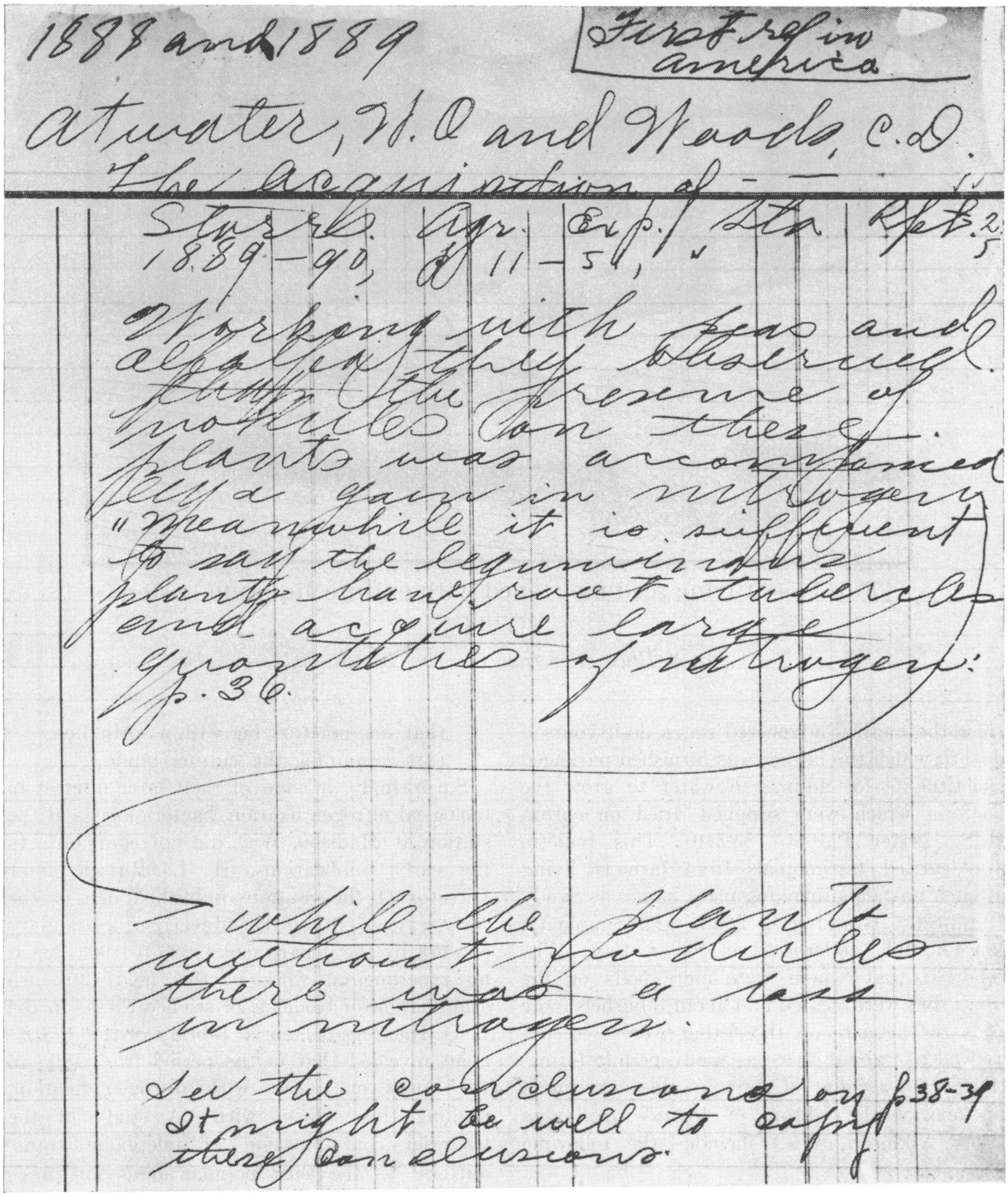
1888 and 1889
First ref in america
Atwater, W. O and Woods, C. D.
The acquisition of —
Storrs Agr. Exp. Sta Rpt. 2
1889–90, p 11–54,
Working with peas and alfalfa they observed ~~that~~ the presence of nodules on these plants was accompanied by a gain in nitrogen
"Meanwhile it is sufficient to say the leguminous plants have root tubercles and acquire large quantities of nitrogen": p. 36.
while the plants without nodules there was a loss in nitrogen.
See the conclusions on p 38–39
It might be well to copy these conclusions

FIG. 5. *E. B. Fred's notes on the later experiments of Atwater and Woods. The comment in the upper right hand corner must refer to confirmation of the results of Hellriegel and Wilfarth.*

evident until young E. B. Fred joined the staff in 1913, replacing Hoffmann who had left. At the Blacksburg station in his native Virginia, Fred had published several reports on nitrogen fixation; his first at Wisconsin appeared in 1916 (12, 13). But during this time he was planning the monograph about which Bay (7) was later to state:

> Easily the most outstanding of all works on economic bacteriology is Hellriegel and Wilfarth's *Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen* (1888), which opened our knowledge to the fundamental supply of nitrogen for the production of protoplasm in plant organs by bacterial action. This subject has been treated in a masterly manner by Fred, Baldwin and McCoy, in their monograph, *Root nodule bacteria and leguminous plants* (1932), an American classic.

Figure 5 reproduces one of Fred's earliest notes used in preparing this outstanding work—his comments on the 1888-1889 papers of Atwater and Woods. Although off to a late start, the "school" founded, and led for many years, by Fred was noted for its balanced program of basic and applied research. Such fundamental studies as the physiology and taxonomy of the bacteria, the cytology of the nodules, and the biochemistry of the nitrogen-fixation process were made in the laboratory and greenhouse along with the field trials. Indeed, one of the first contributions was the production and testing of a reliable inoculum for the farmers—during World War I as part of the "Food Will Win the War" campaign (1). Few of us who underwent training in this school escaped our tour of duty in this service to agriculture. Discontinued during World War II when the department ran low on personnel and agar, it survives in the several directors of laboratories in the firms supplying commercial inoculants. We were young, so it was all exciting and rewarding to those who participated, and I believe that, perhaps, this is what Sam Johnson and Wilbur Atwater had in mind when they dreamed of their ideal station.

Literature Cited

1. Anonymous. 1918. Starting a farm nitrogen factory. The Wisconsin Farmer, March 7, 1918.
2. Atkinson, G. F. 1899. A preliminary report upon the life history and metamorphoses of a root-gall nematode, *Heterodera radicicola* (Greeff) Müll., and the injuries caused by it upon the roots of various plants. Alabama Agr. and Mech. Coll., Agr. Expt. Sta. Bull. No. 9.
3. Atkinson, G. F. 1893. Contribution to the biology of the organism causing leguminous tubercles. Botan. Gaz. **18**:157–166; 226–237; 257–266.
4. Atwater, W. O. 1885. On the acquisition of atmospheric nitrogen by plants. Am. Chem. J. **6**:365–388.
5. Atwater, W. O. 1886. III. On the liberation of nitrogen from its compounds and the acquisition of atmospheric nitrogen by plants. Am. Chem. J. **8**:398–420.
6. Atwater, W. O., and C. D. Woods. 1889. The acquisition of atmospheric nitrogen by plants. Conn. Agr. Expt. Sta. (Storrs), Ann. Rept. **2**:11–51.
7. Bay, J. 1948. Some vital books in science: 1848–1947. Science **107**:485–491.
8. Cottrell, H. M., D. H. Otis, and J. G. Haney. 1900. Soil inoculation for soybeans. Kansas State Coll. Expt. Sta. Bull. 96.
9. Dodson, W. R. 1897. Leguminous root tubercles. Louisiana State Univ., Agr. Expt. Sta. Bull. No. 46.
10. Duggar, J. F. 1897. Soil inoculation for leguminous plants. Alabama Agr. Mech. Coll., Agr. Expt. Sta. Bull. No. 87.
11. Fred, E. B., I. L. Baldwin, and E. M. McCoy. 1932. Root nodule bacteria and leguminous plants. Wisconsin Univ. Studies in Science No. 5.
12. Fred, E. B., and E. J. Graul. 1916. The effect of soluble nitrogenous salts on nodule formation. J. Am. Soc. Agron. **8**:316–328.
13. Fred, E. B., and E. J. Graul. 1916. The gain in nitrogen from growth of legumes on acid soils. Wisconsin Univ. Agr. Expt. Sta. Res. Bull. No. 39.
14. Holland, J. W. 1938. The beginning of public agricultural experimentation in America. Agr. History **12**:271–298.
15. Knoblauch, H. C., E. M. Law, and W. P. Meyer. 1962. State agricultural experiment stations. A history of research policy and procedure. U. S. Dept. Agr. Misc. Publ. No. 904.
16. MacDougal, D. T. 1894. Titles of literature concerning the fixation of free nitrogen by plants. Geological and Natural History Survey of Minnesota. Bull. 9, Botan. Ser. II, p. 186–221.

17. MOORE, G. T. 1905. Soil inoculation for legumes. U.S. Dept. Agr. Bur. Plant Ind. Bull. No. 71.
18. MOORE, G. T., AND T. R. ROBINSON. 1905. Beneficial bacteria for leguminous crops. U.S. Dept. Agr. Farmers' Bull. No. 214.
19. RUSSELL, H. L. 1894. Noteworthy anatomical and physiological researches. The fixation of free nitrogen by plants. Botan. Gaz. **19**:284–293.
20. RUSSELL, H. L. 1904. Summary of experimental work in bacteriology for the years 1893–1903. Abstracted from the Wisconsin Univ. Agr. Expt. Sta. Ann. Rept. 20.
21. SCHNEIDER, A. 1893. Recent investigations concerning rhizobia and free nitrogen assimilation. Agr. Sci. **7**:549–556.
22. TRUE, A. C. 1929. A history of agricultural education in the United States 1785–1925. U.S. Dept. Agr. Misc. Publ. No. 36.
23. TRUE, A. C. 1937. A history of agricultural experimentation and research in the United States 1607–1925. U.S. Dept. Agr. Misc. Publ. No. 251.